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(54) Title: MATERIAL AND METHOD FOR PROMOTING TISSUE GROWTH

(57) Abstract: A composition for promoting tissue growth which includes a material for placing in contact with animal tissue.

MATERIAL AND METHOD FOR PROMOTING TISSUE GROWTH

TECHNICAL FIELD OF THE DISCLOSURE

The present invention generally relates to a material and associated method for promoting tissue growth. The present invention particularly relates to a nanostructured material for promoting tissue growth and an associated method which utilizes the nanostructured material for promoting tissue growth.

BACKGROUND OF THE DISCLOSURE

Tissue replacement materials (e.g., graft and implant materials) for tissues such as cardiovascular, tendon, muscle, bone, bladder, and heart, have attained considerable clinical and economic significance in modern medicine. For example, it is estimated that in 1986 $130 million was spent for vascular grafts alone, not including coronary artery bypass grafts.

Presently, there are two major approaches for the design and production of tissue replacement materials. One approach relies upon tissue replacement materials derived from natural sources. In particular, this approach utilizes various animal tissues as the tissue replacement material. However, a continuing problem with this approach is the inherent risk of transmitting an infectious disease associated with implanting an animal tissue in a human patient. Additional problems with the above described natural source approach include (i) not being able to acquire the appropriate animal tissue due to a limited supply and (ii) the possibility of ethical considerations associated with utilizing an animal tissue implant in a human being. In light of the above discussion it is apparent that utilizing an animal tissue as a tissue replacement material suffers from several drawbacks.

Another approach for the design and production of tissue replacement materials relies upon the use of synthetic substances, such as polytetrafluoroethylene and Dacron®. However, this approach also suffers from several drawbacks. For example, tissue replacement materials fabricated from synthetic substances do not sufficiently biomimic juxtaposed natural tissue and consequently, post-implantation, often fail to properly incorporate or integrate into the natural tissue. In addition, the mechanical characteristics (e.g., elasticity) of these tissue replacement materials are significantly different from the mechanical characteristics of the juxtaposed natural

tissue. These differences can cause mechanical stresses between the tissue replacement material and the juxtaposed natural material, which in turn can lead to implant failure. As such, it is apparent that tissue replacement materials fabricated from synthetic substances also suffer from several drawbacks.

Therefore, in light of the above discussion, it is apparent that what is needed is a tissue replacement composition and an associated tissue replacement method that addresses one or more of the above discussed drawbacks.

SUMMARY OF THE DISCLOSURE

In accordance with one embodiment of the disclosure, a material is provided. The material includes a synthetic organic polymer having a sterile surface with physical structures extending therefrom or defined therein. Each of a substantial number of the physical structures is defined by a set of dimensions where a first dimension of the set is equal to, or less than, about 100 nm and a second dimension of the set is from about 100 nm to about 1 nm.

In accordance with another embodiment of the disclosure, a material is provided. The material includes a synthetic organic polymer having a sterile surface with physical structures extending therefrom or defined therein. Each of a substantial number of the physical structures is defined by a set of dimensions where each dimension of the set is equal to from about 100 nm to about 1 nm.

In accordance with still another embodiment of the disclosure, a material is provided. The material includes a synthetic organic polymer having a sterile surface with physical structures extending therefrom or defined therein. Each of a substantial number of the physical structures is defined by a set of dimensions where each dimension of the set is equal to, or less than, about 100 nm and at least one dimension of the set is from about 1 nm to about 100 nm.

In accordance with yet another embodiment of the disclosure, a method of fabricating a material is provided. The method includes treating a surface of a synthetic organic polymer to cause the creation of physical structures extending from, or defined in, the surface, wherein each of a substantial number of the created physical structures is defined by a set of dimensions where a first dimension of the set is equal to, or less than, about 100 nm and a second dimension of the set is from about

100 nm to about 1 nm. The method also includes sterilizing the surface of the synthetic organic polymer.

In accordance with still another embodiment of the disclosure, a method of fabricating a material is provided. The method includes treating a surface of a synthetic organic polymer to cause the creation of physical structures extending from, or defined in, the surface, wherein each of a substantial number of the physical structures is defined by a set of dimensions where each dimension of the set is equal to from about 100 nm to about 1 nm. The method also includes sterilizing the surface of the synthetic organic polymer.

In accordance with yet another embodiment of the disclosure, a method of fabricating a material is provided. The method includes treating a surface of a synthetic organic polymer to cause the creation of physical structures extending from, or defined in, the surface, wherein each of a substantial number of the physical structures is defined by a set of dimensions where each dimension of the set is equal to, or less than, about 100 nm, and at least one dimension of the set is from about 1 nm to about 100 nm. The method also includes sterilizing the surface of the synthetic organic polymer.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a scanning electron micrograph of a sample of poly-L-lactic/poly-glycolic acid copolymer (note that the bar = 100μm);

FIG. 2 is a scanning electron micrograph of a sample of poly-L-lactic/poly-glycolic acid copolymer after treating the sample with 0.1 N NaOH for 10 minutes (note that the bar = 100μm);

FIG. 3 is a scanning electron micrograph of a sample of poly-L-lactic/poly-glycolic acid copolymer after treating the sample with 5 N NaOH for 30 minutes (note that the bar = 100μm);

FIG. 4 is a scanning electron micrograph of a sample of poly-L-lactic/poly-glycolic acid copolymer after treating the sample with 10 N NaOH for 60 minutes (note that the bar = 100μm);

FIG. 5 is a histogram showing adhesion of bladder smooth muscle cells to treated poly-L-lactic/poly-glycolic acid copolymer samples in comparison

with adhesion to glass and untreated poly-L-lactic/poly-glycolic acid copolymer sample references;

FIG. 6 is a histogram showing adhesion of arterial smooth muscle cells to treated poly-L-lactic/poly-glycolic acid copolymer samples in comparison with adhesion to an untreated poly-L-lactic/poly-glycolic acid copolymer sample reference;

FIG. 7 is a histogram showing bladder smooth muscle cell proliferation on treated poly-L-lactic/poly-glycolic acid copolymer samples in comparison with proliferation on glass and untreated poly-L-lactic/poly-glycolic acid copolymer sample references;

FIG. 8 is a histogram showing adhesion of bladder smooth muscle cells to poly-L-lactic/poly-glycolic acid copolymer samples having different proteins disposed thereon;

FIG. 9 is a histogram showing adhesion of arterial smooth muscle cells to poly-L-lactic/poly-glycolic acid copolymer samples having different proteins disposed thereon;

FIG. 10 is a histogram showing adhesion of osteoblasts (□) and chondrocytes (■) to treated poly-L-lactic/poly-glycolic acid copolymer samples in comparison with adhesion to glass and untreated poly-L-lactic/poly-glycolic acid copolymer sample references;

FIG. 11 is a scanning electron micrograph of a sample of polyurethane (note that the bar = 1μm);

FIG. 12 is a scanning electron micrograph of a sample of polyurethane after treating the sample with 0.1 N HNO3 for 10 minutes (note that the bar = 1μm);

FIG. 13 is a scanning electron micrograph of a sample of polyurethane after treating the sample with 10 N HNO3 for 30 minutes (note that the bar = 1μm);

FIG. 14 is a histogram showing adhesion of bladder smooth muscle cells to treated polyurethane samples in comparison with adhesion to glass and untreated polyurethane sample references;

FIG. 15 is a scanning electron micrograph of a sample of polycaprolactone (note that the bar = 10μm);

FIG. 16 is a scanning electron micrograph of a sample of polycaprolactone after treating the sample with 0.1 N NaOH for 1 minute (note that the bar = 10μm); and

FIG. 17 is a scanning electron micrograph of a sample of polycaprolactone after treating the sample with 10 N NaOH for 10 minutes (note that the bar = 10μm).

DETAILED DESCRIPTION OF THE DISCLOSURE

While the invention is susceptible to various modifications and alternative forms, a specific embodiment thereof has been shown by way of example in the drawings and will herein be described in detail. It should be understood, however, that there is no intent to limit the invention to the particular form disclosed, but on the contrary, the intention is to cover all modifications, equivalents, and alternatives falling within the spirit and scope of the invention as defined by the appended claims.

Materials having enhanced animal tissue compatibility are disclosed herein. The materials include a synthetic organic polymer having a sterile surface with physical structures extending therefrom or defined therein. Each of a substantial number of the physical structures is defined by a set of dimensions (including, for example, length, diameter, width, height, parameter, radius, circumference, and depth) where a first dimension of the set is equal to, or less than, about 100 nm and a second dimension of the set is from about 100 nm to about 1 nm.

In another embodiment, it is contemplated that each of a substantial number of the physical structures is defined by a set of dimensions where each dimension of the set is equal to from about 100 nm to about 1 nm. For example, each of the dimensions is equal to from about 90 nm to about 1 nm, or from about 80 nm to about 1 nm, or from about 70 nm to about 1 nm, or from about 60 nm to about 1 nm, or from about 50 nm to about 1 nm, or from about 40 nm to about 1 nm, or from about 30 nm to about 1 nm, or from about 20 nm to about 1 nm, or from about 10 nm to about 1 nm. In another example, each of the dimensions is equal to from about 90 nm to about 10 nm, or from about 90 nm to about 20 nm, or from about 90 nm to about 30 nm, or from about 90 nm to about 40 nm, or from about 90 nm to about 50 nm, or from about 90 nm to about 60 nm, or from about 90 nm to about 70 nm, or

from about 90 nm to about 80 nm. Furthermore, it should be appreciated that the above described dimension size ranges only serve an illustrative purpose and are not meant to limit the present disclosure. For example, other dimension size ranges within the aforementioned size range from about 100 nm to about 1 nm are contemplated, such as from about 70 nm to about 20 nm, or from about 85 nm to about 15 nm, and the like.

In another embodiment, it is contemplated that each of a substantial number of the physical structures is defined by a set of dimensions where each dimension of the set is equal to, or less than, about 100 nm and at least one dimension of the set is from about 1 nm to about 100 nm.

Note that it is contemplated that the embodiments of the above described sterile surfaces can define the entire surface of a material described herein, or only a portion of the surface of a material described herein.

It should be understood that, as used herein, " physical structure" includes, but is not limited to, for example, protrusions, ridges, depressions, grooves, and wells disposed on, or in, the sterile surface. It should also be understood that what is meant herein by the phrase "the physical structures is defined by a set of dimensions" is that a set of dimensions such as, combinations of dimensions selected from the group including, for example, length, diameter, width, height, parameter, radius, circumference, and depth define the physical structure. For example, a ridge disposed on a surface of a material described herein will have a length dimension, a width dimension, and a height dimension which defines this physical structure and this set of dimensions satisfies the criteria of one or more of the above described sets of dimensions (e.g., (i) a set of dimensions where a first dimension of the set is equal to, or less than, about 100 nm and a second dimension of the set is from about 100 nm to about 1 nm, (ii) a set of dimensions where each dimension of the set is equal to from about 100 nm to about 1 nm, or (iii) a set of dimensions where each dimension of the set is equal to, or less than, about 100 nm, and at least one dimension of the set is from about 1 nm to about 100 nm).

It should also be understood that what is meant herein by "a substantial number of the physical structures" is that the sterile surface has extending therefrom or defined therein significantly more than a deminimis amount of physical structures defined by one or more of the above described sets of dimensions. What is meant

herein by a deminimis amount is an amount of the physical structures defined by one or more of the above described sets of dimensions which would normally be present on a sterile surface as a result of the surface being exposed to its ambient surroundings during, for example, packaging and use thereof. In addition, what is meant herein by "a substantial number of physical structures" includes a sterile surface that has a number or amount of the physical structures defined by the above described set of dimensions effective to increase the tissue compatibility of the sterile surface. For example, as discussed in greater detail below, having a substantial number of physical structures defined by the above described set of dimensions extending from, or defined in, the sterile surface increases animal cell adherence and proliferation on the surface.

It should be appreciated that the material disclosed herein includes synthetic organic polymers. It should be understood that what is meant herein by the phrase "synthetic organic polymers" is an organic polymer that is produced by an artificial rather than a natural process. The synthetic organic polymers included in the material disclosed herein should be cytocompatible. In addition, the synthetic organic polymers can be bioabsorbable and/or bioerodable. The synthetic organic polymers should also be non-toxic, non-carcinogenic, and cause no adverse immunologic response. Representative useful synthetic organic polymers which can be included in the materials disclosed herein include: polyurethane; polyfumarates; polylactides; polyglycolides; polycaprolactones; polyanhydrides; pyrollidones, for example, methylpyrollidone; cellulosic polymers, for example, carboxymethyl cellulose; methacrylates; collagens, for example, gelatin; glycerin and polylactic acid. Synthetic resins, may also be used, including, for example, epoxy resins, polycarbonates, silicones, polyesters, polyethers, polyolefins, synthetic rubbers, polyurethanes, nylons, polyvinylaromatics, acrylics, polyamides, polyimides, phenolics, polyvinylhalides, polyphenyleneoxide, polyketones and copolymers and blends thereof. Copolymers which can be used in the present invention include both random and block copolymers. Polyolefin resins include polybutylene and polyethylene, such as low density polyethylene, medium density polyethylene, high density polyethylene and ethylene copolymers; polyvinylhalide resins include polyvinyl chloride polymers and copolymers and polyvinylidene chloride polymers and copolymers, fluoropolymers; polyvinylaromatic resins include polystyrene polymers and

copolymers a-methylstyrene polymers and copolymers; acrylate resins include polymers and copolymers of acrylate and methacrylate esters, polyamide resins include nylon 6, nylon 11, and nylon 12, as well as polyamide copolymers and blends thereof; polyester resins include polyalkylene terephthalates, such as polyethylene terephthalate and polybutylene terephthalate, as well as polyester copolymers; synthetic rubbers include styrene-butadience and acrylunitrile-butadience-styrene coploymers and; polyketones include polyethertones and polyetherketones. Note that the synthetic organic polymer of the present invention are preferably made from polyurethane and/or the biodegradable polymers of polycaprolactone, poly-L-lactic acid, and poly-glycolic acid, as well as the copolymers of poly-L-lactic acid and poly-glycolic acid, since their mechanical properties are similar to collagen present in soft tissue (such as bladder and cardiovascular tissue).

As indicated above, the surface of a material described herein for placing in contact with animal tissue is sterile. The surface of the material may be sterilized using any appropriate conventional sterilization technique as long as it does not significantly weaken the mechanical strength and mechanical properties of the material nor adversely alter the dimensions of the physical structures extending from or defined in the surface. In particular, the sterilization technique does not alter the dimensions of the physical structures in a way that results in a substantial number of the physical structures failing to satisfy the criteria of one or more of the above described sets of dimensions (e.g., the surface of the material no longer has substantial number of physical structures which are defined by (i) a set of dimensions where a first dimension of the set is equal to, or less than, about 100 nm and a second dimension of the set is from about 100 nm to about 1 nm, (ii) a set of dimensions where each dimension of the set is equal to from about 100 nm to about 1 nm, or (iii) a set of dimensions where each dimension of the set is equal to, or less than, about 100 nm, and at least one dimension of the set is from about 1 nm to about 100 nm). For example, the surface of a material described herein can be sterilized by ethanol soaking (in 90% ethanol for 24 hours) and/or UV light exposure for 12 hours.

It should be appreciated that a material described herein can be fabricated, for example, by (i) treating a surface of a synthetic organic polymer to cause the creation of physical structures extending from, or defined in, the surface, where each of a substantial number of the created physical structures is defined by a

set of dimensions where a first dimension of the set is equal to, or less than, about 100 nm and a second dimension of the set is from about 100 nm to about 1 nm and (ii) sterilizing the surface of the synthetic organic polymer. It should be appreciated that a material described herein can also be fabricated, for example, by (i) treating a surface of a synthetic organic polymer to cause the creation of physical structures extending from, or defined in, the surface, where each of a substantial number of the physical structures is defined by a set of dimensions and where each dimension of the set is equal to from about 100 nm to about 1 nm and (ii) sterilizing the surface of the synthetic organic polymer. It should further be appreciated that a material described herein can be fabricated, for example, by (i) treating a surface of a synthetic organic polymer to cause the creation of physical structures extending from, or defined in, the surface, where each of a substantial number of the physical structures is defined by a set of dimensions and where each dimension of the set is equal to, or less than, about 100 nm, and at least one dimension of the set is from about 1 nm to about 100 nm and (ii) sterilizing the surface of the synthetic organic polymer.

It should further be appreciated a material described herein can be fabricated, for example, by (i) substantially increasing the number of the above described physical structures extending from or defined in a surface of a synthetic organic polymer or (ii) creating a substantial number of the above described physical structures extending from or defined in a surface of a synthetic organic polymer which only possessed a diminimus number of such physical structures.

Now turning to the fabrication of an exemplary material of the present invention, polymer pellets (0.5 g) are dissolved in 8 ml of an appropriate solvent (for example, chloroform for poly-lactic/glycolic acid, and tetrahydrofuran for polyurethane and polycaprolactone), vortexed, cast, vacuum dried at room temperature for 48 hours, and then cut into three-dimensional scaffolds (1 cm x 1 cm x 0.5 cm). Synthetic organic polymers with basic functional groups (such as polyurethane) are soaked in acidic solutions (e.g., a HNO_3 solution) having a concentration of about, for example 0.1 N to about 10N of for a time period of about, for example, 15 minutes to about 5 hours, while shaking at room temperature. In contrast, synthetic organic polymers with acidic functional groups (such as poly-L-lactic acid, poly-glycolic acid, polycaprolactone and their copolymers) can be soaked in basic solutions, for example NaOH, having a concentration of about, for example,

0.1 N to about 10 N for a time period of about, for example 15 minutes to about 5 hours, while shaking at room temperature.

A sample of a poly-L-lactic acid/poly-glycolic acid copolymer (hereinafter referred to as PLGA) was subjected to the above described procedure. In particular, the 0.5 g of PLGA (purchased from Polysciences, Cat. No. 23986) was dissolved in chloroform, vortexed, cast, vacuum dried at room temperature for 48 hours, and then cut into three-dimensional scaffolds (1 cm x 1 cm x 0.5 cm). The PLGA scaffolds were then divided into four separate samples, i.e., a control sample, a sample A, a sample B, and a sample C. The control sample was not subjected to any further treatments. However, samples A, B, and C were subjected to the following treatments. The sample A scaffold was soaked in a 0.1 N NaOH solution for ten minutes. After ten minutes the NaOH solution was neutralized with an acid such as HCl. The NaOH solution was then evaporated under reduced pressure to obtain a sheet of synthetic organic polymer material composed of PLGA. The sample B scaffold was soaked in a 5 N NaOH solution for 30 minutes. After the 30-minute time period the NaOH solution was neutralized with an acid such as HCl. The NaOH solution was then evaporated under reduced pressure to obtain a sheet of synthetic organic polymer material composed of PLGA. Sample C was soaked in a 10 N NaOH solution for 60 minutes, after which the NaOH solution was neutralized with an acid such as HCl. The NaOH solution was then evaporated under reduced pressure to obtain a sheet of synthetic organic polymer material composed of PLGA.

As shown in FIG. 1 the control sample of PLGA scaffold, which was not treated with NaOH, has a number of physical structures on the surface thereof having dimensions of about 10 μm to about 15 μm (i.e., $d \approx 10$ μm - 15 μm; note that the bar in the lower right corner of each scanning electron micrograph equals 100 μm). However as shown in FIG. 2, sample A of the PLGA scaffold, which was treated with 0.1 N NaOH for ten minutes, has a number of physical structures on the surface thereof having dimensions of about 5 μm to about 10 μm (i.e., $d \approx 5$ μm - 10 μm). Also as shown in FIG. 3, sample B of the PLGA scaffold, which was treated with the 5 N NaOH for thirty minutes, results in sample B having on the surface thereof a number of physical structures having dimensions of about 100 nm to about 10 μm (i.e., $d \approx 100$ nm - 10 μm). Furthermore, as shown in FIG. 4, sample C of the

PLGA scaffold, which was treated with the 10 N NaOH for 60 minutes, has a number of physical structures on the surface thereof having dimensions of about 50 nm to about 100 nm (i.e., d ≈ 50 nm - 100 nm).

The surfaces of the above described control and samples were evaluated according to according to standard scanning electron microscope (SEM) techniques (Webster TJ, Siegel RW, Bizios R. Osteoblast adhesion on nanophase ceramics. Biomaterials 1999; 20: 1221-1227, incorporated herein by reference) using a JEOL JSM-840 SEM (JEOL USA, Inc., Peabody, MA). Briefly, the control and samples were sputter-coated in gold-palladium in the presence of argon gas at room temperature. These coated samples were then visualized at low (250X or 500X), medium (1000X), and high (5000X) magnifications at an accelerating voltage of 5 kV. Dimensions of physical structures on or in the surface of the sample were measured from the SEM images using Adobe Photoshop (Adobe Systems, Inc.) imaging software; physical structure dimensions are reported as ranges and were determined by averaging five different measurements per sample.

Still referring to FIGS. 1-4 it should be understood that treating PLGA in the above described manner results in the physical structures being substantially aligned in a particular direction. In particular as shown in FIG. 2, the physical structures of sample A of the PLGA scaffold, are substantially aligned in the direction indicated by the arrow 10. As shown in FIG. 3, the physical structures of sample B of the PLGA scaffold, are substantially aligned in the direction indicated by the arrow 12. As shown in FIG. 4, the physical structures of sample C of the PLGA scaffold, are substantially aligned in the direction indicated by the arrow 14. It should be understood that the above-described alignment of the physical structures is an advantage of the present invention since such alignment further biomimics the natural proteins that make up natural animal soft tissue, such as bladder, bone, cardiovascular, tendon, and muscle tissues.

It should be appreciated that the ability of a cell to adhere to a material is important to the success of any material to be used to promote tissue growth. In particular, the ability of anchorage-dependant cells (e.g., smooth muscle cells) to adhere to a material is important factor for subsequent cell functioning, such as tissue growth and/or organ regeneration. The below described cell adhesion experiments were conducted as follows. Cells were suspended in recommended media

supplemented with serum. The cells were then separately seeded (2,500 cell/cm^3) on/in the synthetic organic polymer having a substantial number of physical structures on the surface thereof indicated in FIG. 5 and allowed to adhere to the polymer for 4 hours under standard cell culture conditions. After the prescribed time period, non-adherent cells were removed from each polymer by rinsing with phosphate-buffered saline. Adherent cells were fixed, stained, and counted according to standard techniques. Adherent cell density (cells per unit surface area) were determined by averaging the number of cells in five random fields. Adherent cells were visualized, and the images stored, using Image Pro computer software. All experiments were run in triplicate and were repeated at least three separate times per substrate tested. Data was analyzed using standard analysis of variance "ANOVA" techniques with factorial designs.

Now turning to FIG. 5, the data illustrated therein represents the adhesion of bladder smooth muscle cells to the surface of glass (reference substrate), untreated PLGA (control; not treated with NaOH), sample A of PLGA which has, as indicated above, physical structures having dimensions of about 5 μm to about 10 μm, sample B of PLGA which has, as indicated above, physical structures having dimensions of about 100 nm to about 10 μm, and sample C of PLGA scaffold which has, as indicated above, physical structures having dimensions of about 50 nm to about 100 nm. As shown in FIG. 5, bladder smooth muscle cell adhesion was three times greater on sample C as compared to untreated PLGA.

Now turning to FIG. 6, there is shown a similar comparison with the exception that arterial smooth muscle cells were tested for adhesion rather than bladder smooth muscle cells. In particular, the adhesion of arterial smooth muscle cells was tested on untreated PLGA (control; not treated with NaOH), sample A of PLGA which has, as indicated above, physical structures having dimensions of about 5 μm to about 10 μm and sample C of PLGA which has, as indicated above, physical structures having dimensions of about 50 nm to about 100 nm. Similar to the previous data illustrated in FIG. 5, FIG. 6 demonstrates that arterial smooth muscle cells also adhere to samples A and C of the PLGA samples better than untreated PLGA.

Now referring to FIG. 10, there is shown a similar comparison with the exception that chondrocytes (■) and osteoblasts (□) were tested for adhesion rather

than bladder smooth muscle cells or arterial smooth muscle cells. In particular, the adhesion of chondrocytes and osteoblasts was tested on glass, untreated PLGA (labeled conventional in FIG. 10), sample A of PLGA and sample C of PLGA . Similar to the above discussed data, FIG. 10 demonstrates that chondrocytes and osteoblasts adhere to samples A and C of the PLGA better than untreated PLGA.

It should also be appreciated that the ability of a cell to proliferate (that is, divide) on a material can be important to the success of any material to be used to promote tissue growth. The below described cell proliferation experiments were conducted as follows. Bladder smooth muscle cells were suspended in the recommended media and supplemented with serum. The cells were then separately seeded (2,500 cells/cm^3) on/in the substrates indicated in FIG. 7. The cells were then cultured under standard cell culture conditions for 1, 3, and 5 day time periods. At the conclusion of the experiment, the cells were fixed, stained, and counted according to established methods. Cell density (cells per unit surface area) was determined by averaging the number of cells in five random fields.

Now turning to FIG. 7, the data illustrated therein represents the proliferation of bladder smooth muscle cells on the surface of glass (reference substrate), untreated PLGA (control; not treated with NaOH), sample A of PLGA (about 5 μm to about 10 μm dimension physical structures), sample B of PLGA (about 100 nm to about 10 μm dimension physical structures) and sample C of PLGA scaffold (about 50 nm to about 100 nm dimension physical structures). FIG. 7 demonstrates that bladder smooth muscle cells proliferate better on samples A, B, and C of the PLGA scaffolds as compared to untreated PLGA.

In another embodiment of the present disclosure, it is contemplated that extra cellular matrix proteins can be utilized to spaciously control specific cell locations on a material of the present invention. For example, disposing an extracellular protein on the inner and outer portions of a sheet of material of the present invention prior to placing the material in contact with animal tissue can selectively control the growth of cells thereon.

In the present disclosure, proteins that control bladder and arterial smooth muscle adhesion were determined by coating borosilicate glass with 5 micrograms/milliter of specific extracellular matrix proteins. Cells were allowed to adhere for four hours at which time they were fixed, stained, and counted. Now

turning to FIG. 8, the data represented therein demonstrates that, as compared to other proteins such as albumin, gelatin, fibronectin, vitronectin, laminin, collagen type I, and collagen type III, bladder smooth muscle cell adhesion was significantly greater on the material pretreated with collagen type IV after four hours. Furthermore, as shown in FIG. 9, as compared to other proteins such as FBS, albumin, fibronectin, laminin and collagen type I, arterial smooth muscles cell adhesion was significantly greater on material of the present disclosure pretreated with collagen type III after four hours. In this manner, proteins have been identified to control where a cell will adhere on materials described herein. This is important since cells are found in select regions of natural tissue. For example, the materials described herein could be treated before implantation with identified proteins to control where cells will adhere and proliferate once the polymers are implanted.

Now turning to another synthetic organic polymer which can be utilized in a material described herein: polyurethane. A sample of a polyurethane (hereinafter referred to as PU) was subjected to the above described procedure described for PLGA. In particular, the 0.5 g of PU (purchased from Thermedics, Inc.Cat. No. Tecoflex EG-72D) was dissolved in tetrahydrofuran, vortexed, cast, vacuum dried at room temperature for 48 hours, and then cut into three-dimensional scaffolds (1 cm x 1 cm x 0.5 cm). The PU scaffolds were then divided into three separate samples, i.e., a control sample, a sample A and a sample B. The control sample was not subjected to any further treatments. However, samples A and B were subjected to the following treatments. The sample A scaffold was soaked in a 0.1 N HNO_3 solution for ten minutes. After ten minutes the HNO_3 solution was neutralized with a base such as NaOH. The HNO_3 solution was then evaporated under reduced pressure to obtain a sheet of polymer material composed of PU. The sample B scaffold was soaked in a 10 N HNO_3 solution for 30 minutes. After the 30 minute time period the HNO_3 solution was neutralized with a base such as NaOH. The HNO_3 solution was then evaporated under reduced pressure to obtain a sheet of synthetic organic polymer material composed of PU.

As shown in FIG. 11 the control sample of PU scaffold, which was not treated with HNO_3, has a number of physical structures on the surface thereof having dimensions of about 10 μm to about 15 μm (i.e., $d \approx 10$ μm - 15 μm; note that the bar in the lower right corner of each scanning electron micrograph equals 1 μm).

However as shown in FIG. 12, sample A of the PU scaffold, which was treated with the 0.1 N HNO_3 for ten minutes, has a number of physical structures on the surface thereof having dimensions of about 100 nm to about 1 μm (i.e., d ≈ 100 nm - 1 μm). Furthermore, as shown in FIG. 13, sample B of the PU scaffold, which was treated with the 10 N HNO_3 for 30 minutes, has a number of physical structures on the surface thereof having dimensions of about 50 nm to about 100 nm (i.e., d ≈ 50 nm - 100 nm).

Now turning to FIG. 14, the data illustrated therein represents the adhesion of bladder smooth muscle cells to the surface of glass (reference substrate), untreated PU (conventional; not treated with HNO_3), sample A of PU scaffold (physical structure dimensions of about 1 μm to about 100 nm, and sample B of PU scaffold (physical structure dimensions of about 100 nm to about 50 nm). As shown in FIG. 14, as compared to glass or untreated PU, bladder smooth muscle cell adhesion is greater on samples A and B. For example, bladder smooth muscle cell adhesion was two times greater on sample B as compared to untreated PU.

Now turning to another synthetic organic polymer which can be utilized in a material described herein: polycaprolactone. A sample of a polycaprolactone (hereinafter referred to as PC) was subjected to the above described procedure described for PLGA . In particular, the 0.5 g of PC (purchased from Polysciences, Cat. No. 19561-500) was dissolved in tetrahydrofuran, vortexed, cast, vacuum dried at room temperature for 48 hours, and then cut into three-dimensional scaffolds (1 cm x 1 cm x 0.5 cm). The PC scaffolds were then divided into three separate samples, i.e., a control sample, a sample A and a sample B. The control sample was not subjected to any further treatments. However, samples A and B were subjected to the following treatments. The sample A scaffold was soaked in a 0.1 N NaOH solution for one minute. After one minute the NaOH solution was neutralized with an acid such as HCl. The NaOH solution was then evaporated under reduced pressure to obtain a sheet of polymer material composed of PC. The sample B scaffold was soaked in a 10 N NaOH solution for 10 minutes. After the 10 minute time period the NaOH solution was neutralized with an acid such as HCl. The NaOH solution was then evaporated under reduced pressure to obtain a sheet of polymer material composed of PC.

As shown in FIG. 15 the control sample of PC scaffold, which was not treated with NaOH, has physical structures on the surface thereof having dimensions of about 10 μm to about 15 μm (i.e., d ≈ 10 μm - 15 μm; note that the bar in the lower right corner of each scanning electron micrograph equals 10 μm). However as shown in FIG. 16, sample A of the PC scaffold, which was treated with the 0.1 N NaOH for one minute, has physical structures on the surface thereof having dimensions of about 100 nm to about 10 μm (i.e., d ≈ 100 nm - 10 μm). Furthermore, as shown in FIG. 17, sample B of the PU scaffold, which was treated with the 10 N NaOH for 10 minutes, has physical structures on the surface thereof having dimensions of about 50 nm to about 100 nm (i.e., d ≈ 50 nm - 100 nm).

While the invention has been illustrated and described in detail in the drawings and foregoing description, such illustration and description is to be considered as exemplary and not restrictive in character, it being understood that only the preferred embodiments have been shown and described and that all changes and modifications that come within the spirit of the invention are desired to be protected.

CLAIMS:

1. material, comprising:

a synthetic organic polymer having a sterile surface with physical structures extending therefrom or defined therein, wherein each of a substantial number of said physical structures is defined by a set of dimensions where a first dimension of said set is equal to, or less than, about 100 nm and a second dimension of said set is from about 100 nm to about 1 nm.

2. A material, comprising:

a synthetic organic polymer having a sterile surface with physical structures extending therefrom or defined therein, wherein each of a substantial number of said physical structures is defined by a set of dimensions where each dimension of said set is equal to from about 100 nm to about 1 nm.

3. A material, comprising:

a synthetic organic polymer having a sterile surface with physical structures extending therefrom or defined therein, wherein each of a substantial number of said physical structures is defined by a set of dimensions where each dimension of said set is equal to, or less than, about 100 nm, and at least one dimension of said set is from about 1 nm to about 100 nm.

4. A method of fabricating a material, comprising:

treating a surface of a synthetic organic polymer to cause the creation of physical structures extending from, or defined in, said surface, wherein each of a substantial number of said created physical structures is defined by a set of dimensions where a first dimension of said set is equal to, or less than, about 100 nm and a second dimension of said set is from about 100 nm to about 1 nm; and

sterilizing said surface of said synthetic organic polymer.

5. A method of fabricating a material, comprising:

treating a surface of a synthetic organic polymer to cause the creation of physical structures extending from, or defined in, said surface, wherein each of a substantial number of said physical structures is defined by a set of dimensions where each dimension of said set is equal to from about 100 nm to about 1 nm; and

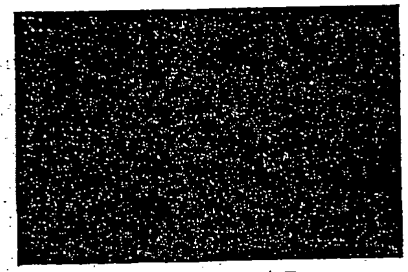
sterilizing said surface of said synthetic organic polymer.

6. A method of fabricating a material, comprising:

treating a surface of a synthetic organic polymer to cause the creation of physical structures extending from, or defined in, said surface, wherein each of a substantial number of said physical structures is defined by a set of dimensions where each dimension of said set is equal to, or less than, about 100 nm, and at least one dimension of said set is from about 1 nm to about 100 nm; and

sterilizing said surface of said synthetic organic polymer.

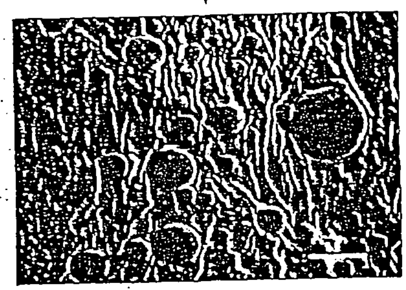
Fig. 1



d ≈ 10 μm – 15 μm

10

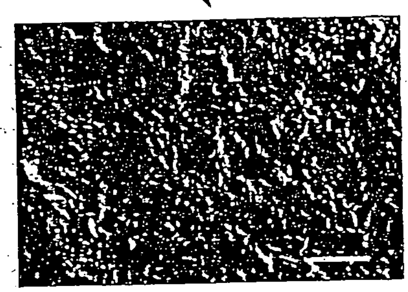
Fig. 2



d ≈ 5 μm – 10 μm

12

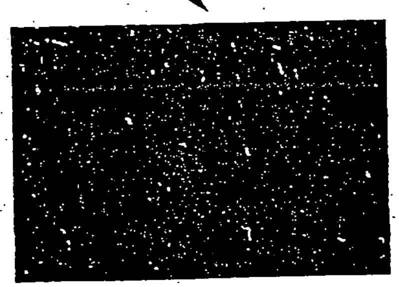
Fig. 3



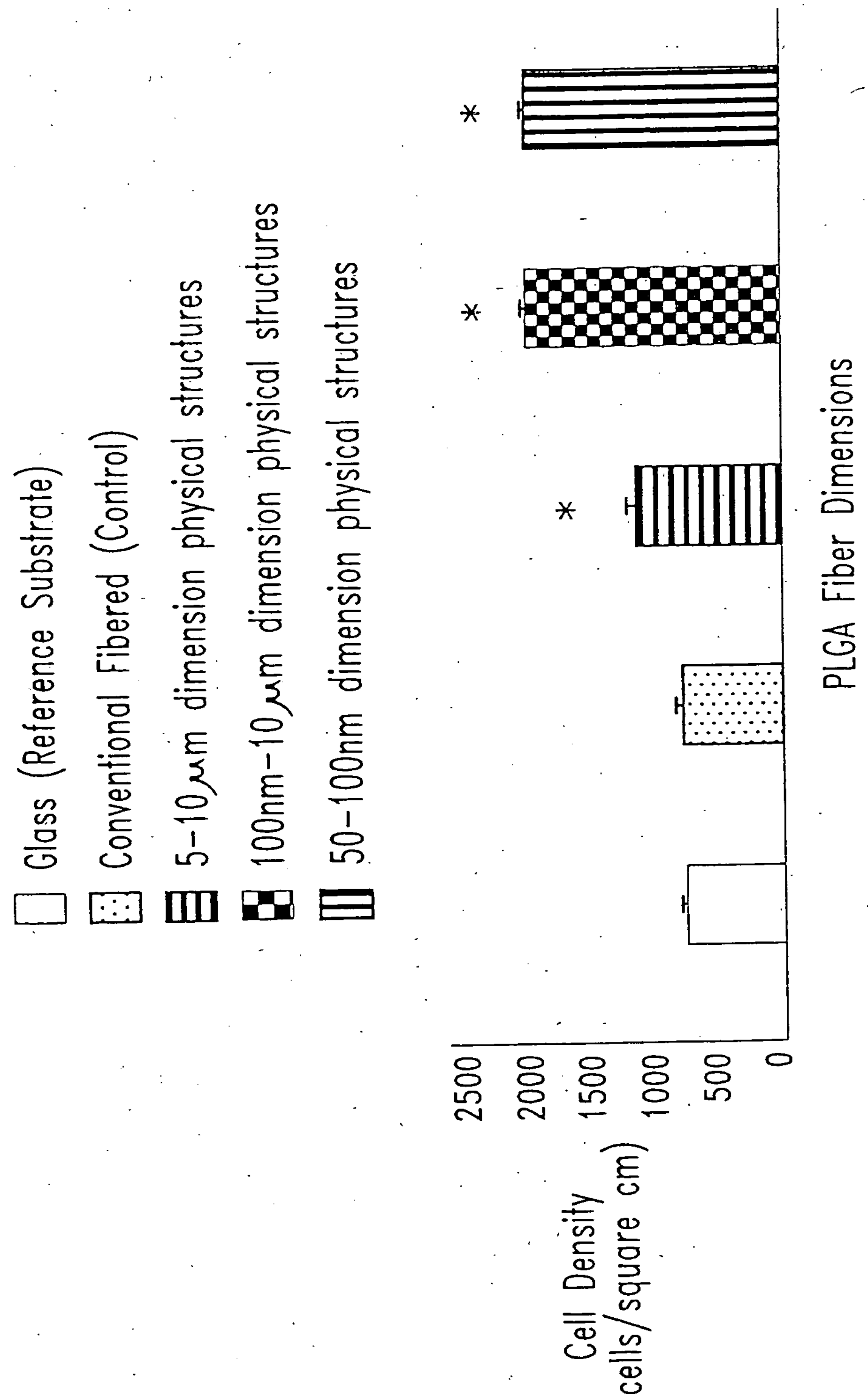
d ≈ 100nm – 10μm

14

Fig. 4



d ≈ 50–100nm



Values are mean +/− SEM; *p <0.01 (compared to conventional fibered PLGA films).

Fig. 5

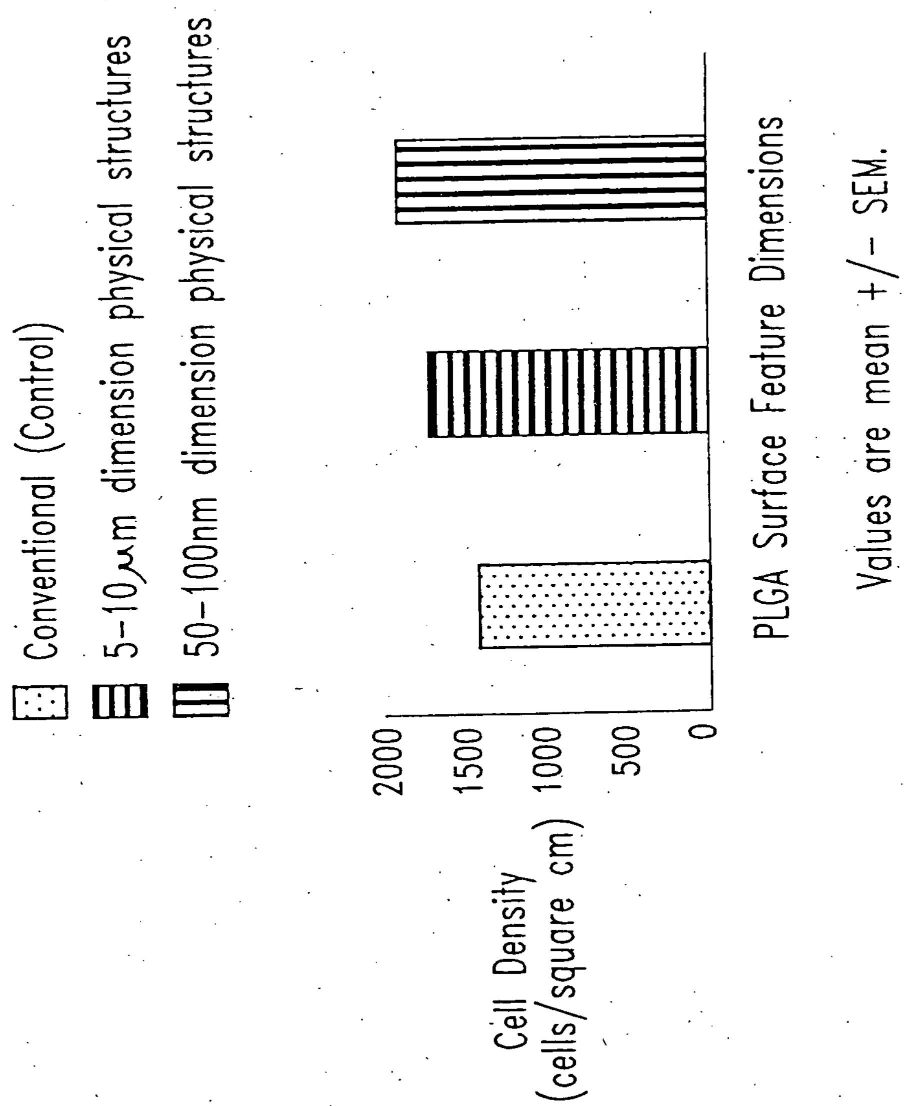
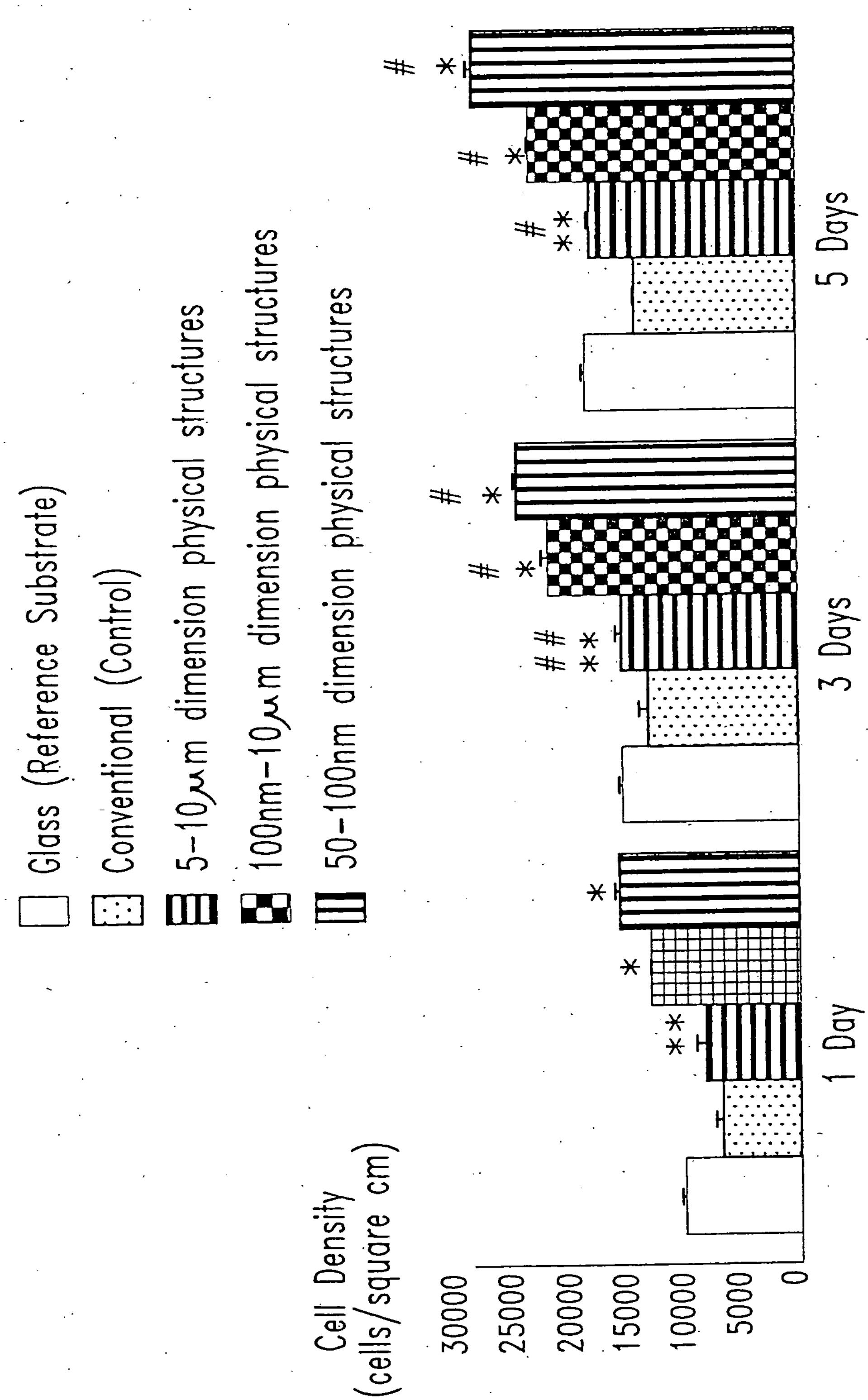
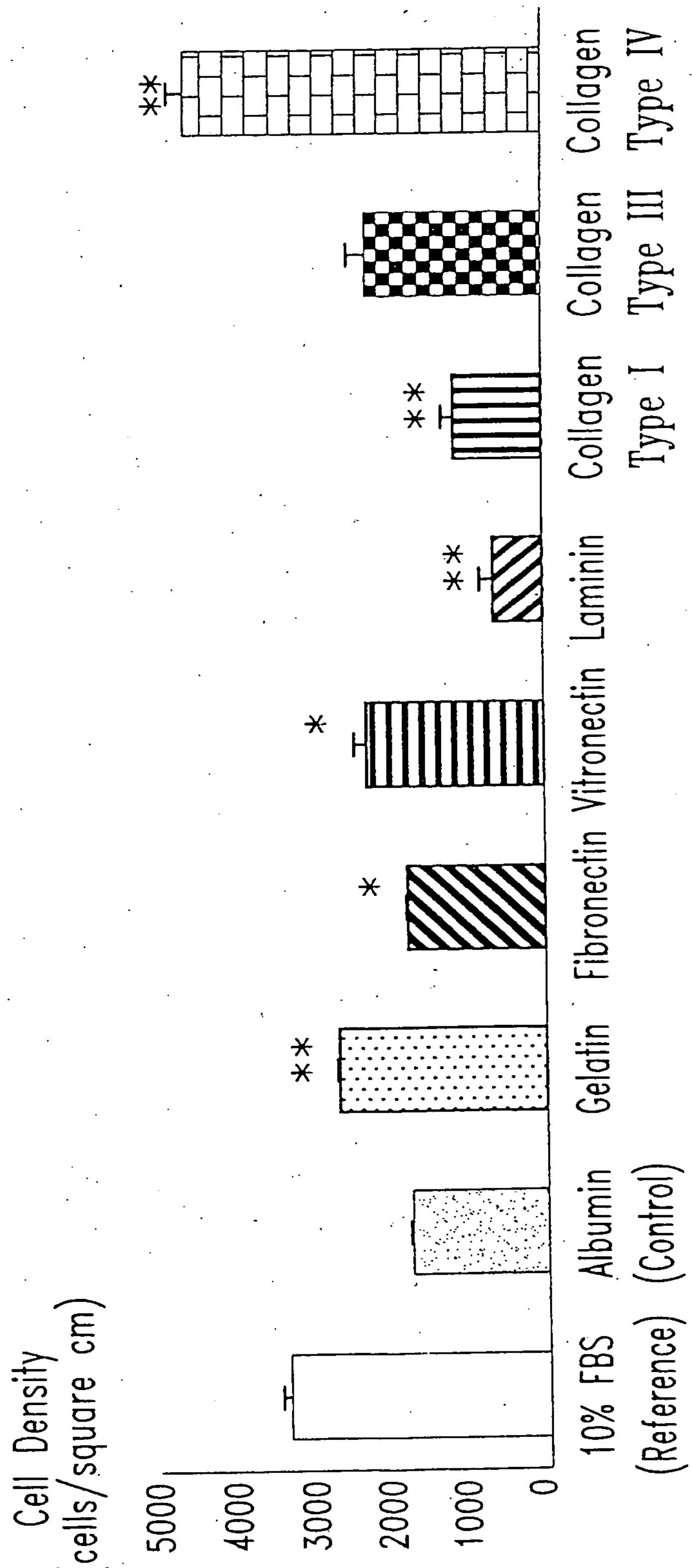

Conventional (Control)
5–10μm dimension physical structures
50–100nm dimension physical structures
Cell Density
(cells/square cm)
2000
1500
1000
500
0
PLGA Surface Feature Dimensions
Values are mean +/– SEM.

Fig. 6



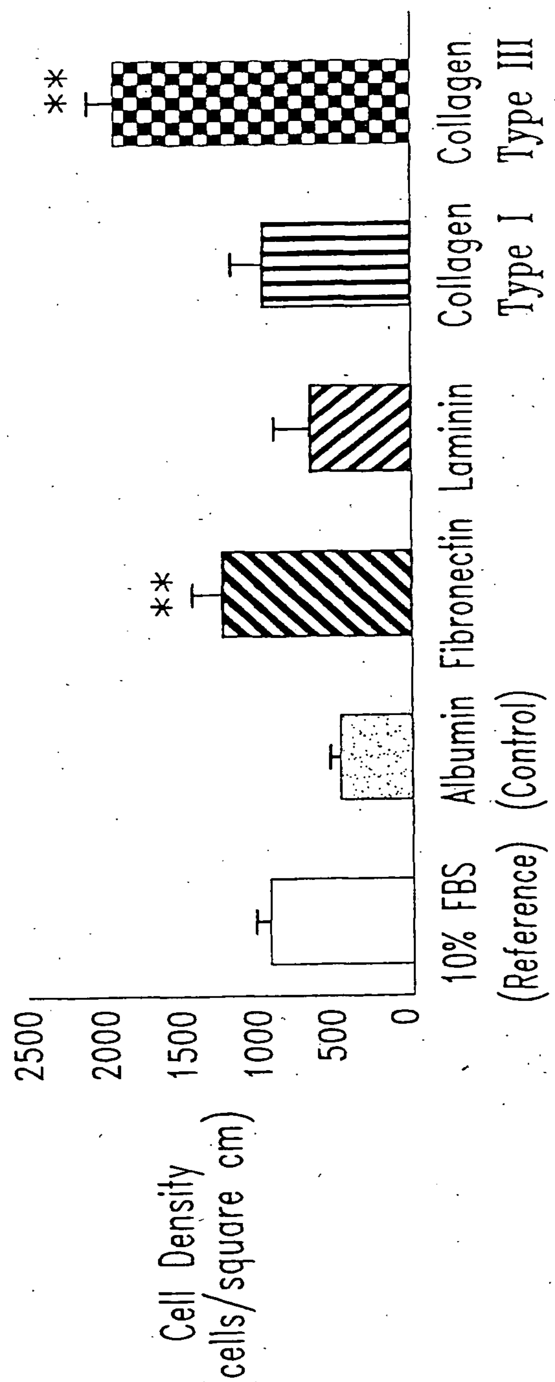
Values are mean +/– SEM; * ρ <0.01 and ** ρ <0.05 compared to controls at respective day.
ρ <0.01 and ## ρ <0.05 compared to respective 1 day samples.

Fig. 7



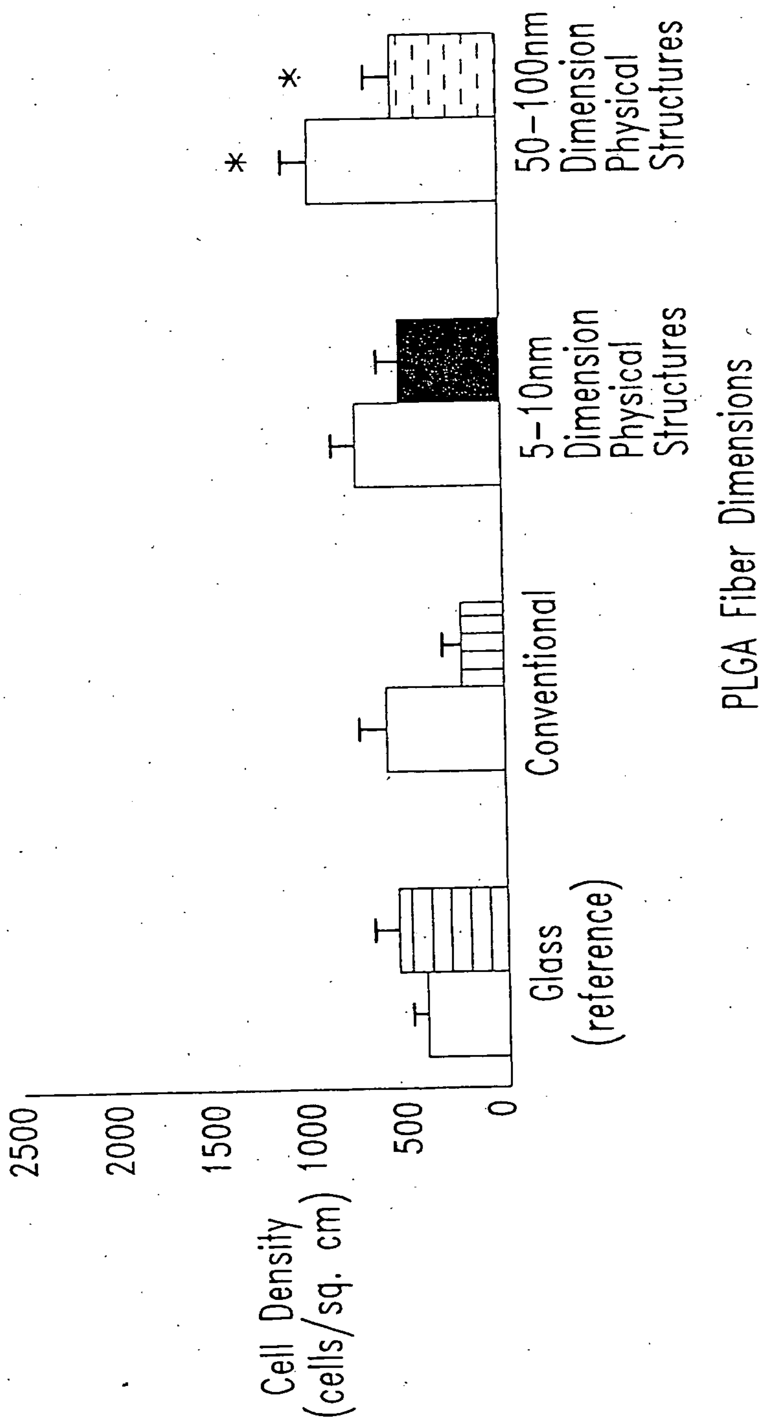
Values are mean +/- SEM; * ρ <0.05 and ** ρ <0.01 compared to controls (albumin coated glass substrates).

Fig. 8



Values are mean +/- SEM; ** ρ <0.01 compared to controls (albumin coated glass substrates).

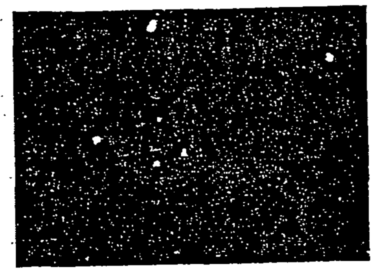
Fig. 9



Values are mean +/- SEM; *ρ<0.1 compared to cell adhesion on untreated PLGA sample.

Fig. 10

Fig. 11

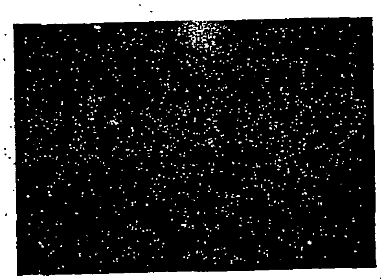


(a) Control (Untreated); d ≅ (10μm – 15μm)

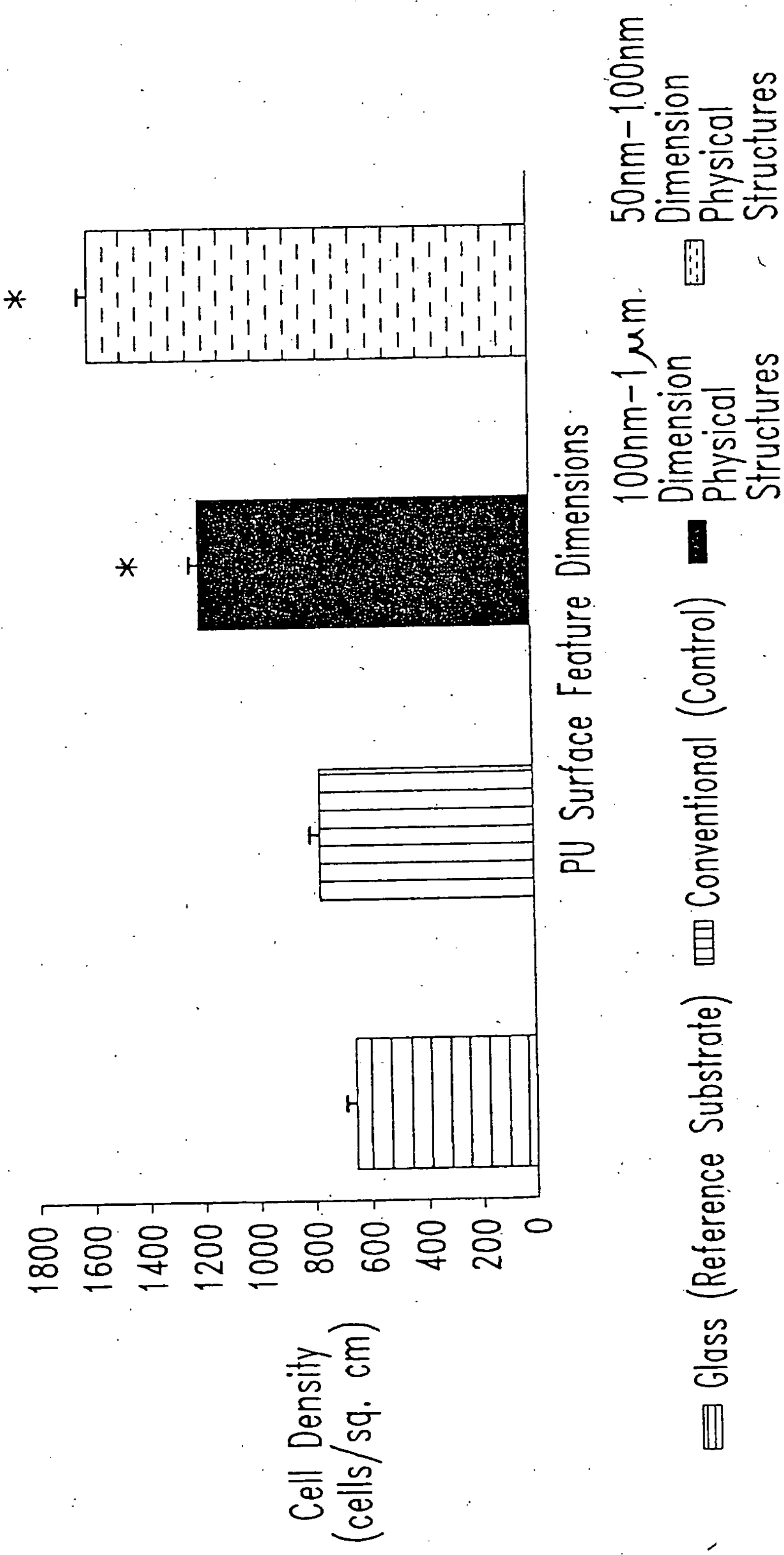
Fig. 12

(b) 0.1N HNO_3 (10 min); d ≅ (100nm – 1μm)

Fig. 13



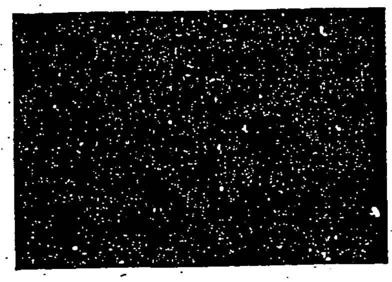
(c) 10N HNO_3 (30 min); d ≅ (50nm – 100nm)



Values are mean +/- SEM; n=3; * ρ<0.01 (compared to cell adhesion on conventional PU films).

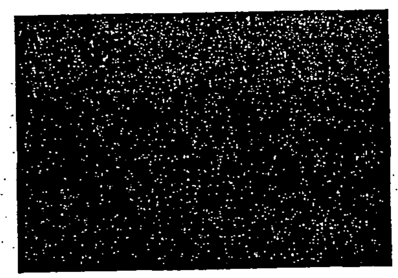
Fig. 14

Fig. 15



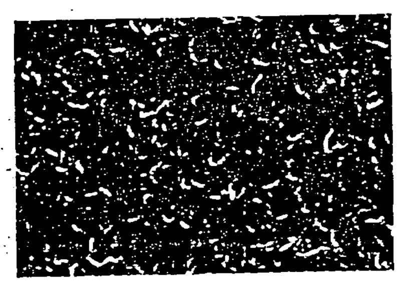
d ≈ 10 μm – 15 μm

Fig. 16



d ≈ 100nm – 10 μm

Fig. 17



d ≈ 50nm – 100nm